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Use of Multivariate Analysis To Compare Antimicrobial Agents on the Basis of In Vitro Activity Data

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Multivariate techniques such as principal component analysis or similar factor analysis help in analyses of the simultaneous interrelationships among several variables. A comparative multivariate analysis on the in vitro activities of eight antimicrobial agents, including the novel molecule daptomycin, is presented. Multivariate analysis detects components or factors and establishes connections among antimicrobial agents on the basis of their different levels of participation in each factor. The first principal component was dominated by vancomycin, teicoplanin, and rifampin (0.94344, 0.92792, and 0.72127, respectively). The second principal component showed strong effects from imipenem, gentamicin, and cephalothin (0.87922, 0.86126, and 0.68870, respectively). Daptomycin stood out alone in the third principal component (0.83983). The first three components defined 81.5% of the total variance and could easily be represented graphically in a three-dimensional scatter plot. In this graphic representation, the eight antimicrobial agents clustered in three different spatial regions; daptomycin occupied a separate spatial position. The use of multivariate analysis offers a different approach to determination of the in vitro activities of new antimicrobial agents and adds some new data on the relationships among different classes. Notwithstanding its limitations, the application of these methods in microbiology and drug development could be an additional tool for use in processing information.

A common way to compare the activities of antimicrobial agents is matching susceptibility data in a standard manner by using MICs for 50% (MIC_{50}) or 90% (MIC_{90}) of isolates tested. MICs show the interaction of one antimicrobial agent with one microorganism. The comparison of two MICs reflects how one antimicrobial agent acts on two different strains or how two antimicrobial agents act on the same strain. This conventional approach is satisfactory in many cases, but it does not depict the interactions of several anti-infective agents with several organisms. Multivariate techniques, such as principal component analysis (PCA) or factor analysis (5), could aid in this comparison.

PCA and factor analysis assist in the identification of the interrelationships of multiple variables, all acting simultaneously. Multivariate methods handle large volumes of data resulting from the interactions of multiple variables and identify principal components or factors that link some of the variables and that unlink others. There are as many components or factors identified as there are variables studied. The information provided by the factors is not identical; the first factors reflect links of a greater strength and influence on the context than do the last factors. The graphic, three-dimensional representation of the principal components helps to provide a better understanding of the interrelations among variables. These relationships can be extrapolated qualitatively to generate postulates or to reveal trends, making multivariate techniques useful hypothesis-generating tools.

These analyses can be useful for comparing new antimicrobial agents with existing ones. Microbiological activity influences the interest in a new antimicrobial agent in its early development stages. In the present study, multivariate analysis techniques were used to investigate the interrelationships of eight different antimicrobial agents including daptomycin (DAP), a new lipopeptide antimicrobial agent (3), on the basis of in vitro data (MIC) obtained for 17 different groups of microorganisms.

MATERIALS AND METHODS

Study selection and microbiology data. A bibliographic search of articles in the MEDLINE database mentioning "daptomycin," "susceptibility," "in vitro," or "activity" during the period 1986 to 1990 was performed by using CD-ROM technology. Only articles that described an in vitro assay in which a dilution technique was used were preselected. DAP needs calcium ions to express its activity both in vivo and in vitro. Thus, to avoid erroneous MICs of daptomycin, studies that did not use cation-supplemented medium (17) were excluded. Noncomparative studies or anecdotal communications were not taken into account. MICs of different antimicrobial agents for different species were tabulated from 16 articles that met the former criteria (4, 7, 8, 10–15, 17–23). Only MIC_{90} data were recorded, disregarding MICs for individual strains.

Data from the different studies were then combined in a manner that had clinical relevance, either by species, genus, group, serotype, or drug susceptibility phenotype. PCA and factor analysis require observations with no missing values, and in the literature collected, different studies used different antimicrobial agents. Only eight drugs were consistently used in 11 studies, providing information on 17 groups of microorganisms (see Table 1). These 17 groups of organisms and eight antimicrobial agents made up the data set from which all the statistical computations were done. Table 1 shows the base-2 logarithm on the weighted mean (*cMIC*) of grouped microorganisms from the different studies considered. This mean was weighted on the basis of the number of strains tested in each study. The base-2 logarithm of this *cMIC* ($\log_2 cMIC$) was then calculated and rounded to the next whole number, that is, a

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TABLE 1. $\log_2$ *cMIC* for antimicrobial agents and species undergoing multivariate analysis

Species (no. of strains)	$\log_2$ *cMIC*								Reference
	DAP	VAN	TEI	CLT	IMI	GEN	CIP	RIF	
E. faecalis (215)	1	2	0	5	1	6	1	3	11–13, 15, 18
Corynebacterium sp. strain JK (133)	0	0	0	6	6	8	1	−2	8, 13, 18
Lactobacillus spp. (45)	0	9	9	4	−3	2	2	5	7, 22
Leuconostoc spp. (60)	−2	9	9	4	3	0	2	3	7, 22
Listeria spp. (88)	2	0	1	2	−3	−2	0	−5	11, 12, 15, 17, 18, 21
Pediococcus spp. (24)	−2	9	9	4	−3	1	4	1	22
S. aureus (methicillin resistant) (131)	−1	0	0	6	5	6	0	0	11–13, 15, 18
S. aureus (methicillin susceptible) (144)	−1	0	0	0	−3	−1	0	−6	11–13, 15, 18
Coagulase-negative staphylococci (methicillin resistant) (80)	−1	1	3	5	5	6	−1	−5	15, 18
Coagulase-negative staphylococci (methicillin susceptible) (39)	0	1	1	−1	−1	4	−1	−6	15, 18
S. epidermidis (methicillin resistant) (95)	0	2	2	0	2	4	−1	−5	11, 13, 20
S. epidermidis (methicillin susceptible) (80)	0	1	1	−2	−2	−4	−2	−4	11, 13, 20
S. haemolyticus (methicillin resistant) (85)	1	2	4	6	6	5	−2	−7	13, 20
S. bovis (39)	−1	0	1	−2	−3	3	2	−2	11, 13, 18
Viridans group streptococci (88)	0	0	−2	0	−2	4	2	−3	11, 13, 15, 18
Group A streptococci (45)	−2	0	−2	−3	−5	4	0	−4	11, 13
Group B streptococci (58)	−2	−1	−2	−2	5	4	0	−2	15, 18

real twofold dilution. As an example, the series of base-2 logarithms −3, −2, −1, 0, 1, 2, 3, 4, and 5 would correspond to the series 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 (expressed in micrograms per milliliter), respectively. For simplicity, when dilutions were expressed as less than or equal to, the dilution became the number itself (i.e., ≤0.25 became 0.25). When dilutions were expressed as greater than, the value considered was the next twofold dilution (i.e., >8 became 16).

The antimicrobial agents included were DAP, vancomycin (VAN), teicoplanin (TEI), ciprofloxacin (CIP), gentamicin (GEN), cephalothin (CLT), rifampin (RIF), and imipenem (IMI). The organisms considered were *Enterococcus faecalis*; *Corynebacterium* sp. strain JK; *Lactobacillus*, *Leuconostoc*, *Listeria*, and *Pediococcus* species; methicillin-susceptible and -resistant *Staphylococcus aureus*; methicillin-susceptible and -resistant coagulase-negative staphylococci; methicillin-susceptible and -resistant *Staphylococcus epidermidis*; methicillin-resistant *Staphylococcus haemolyticus*; *Streptococcus bovis*; group A streptococci; group B streptococci; and viridans group streptococci. The antimicrobial agents and organisms that were studied originally and that were excluded from the present analysis because of missing data were the following: cefotaxime, clindamycin, oxacillin, penicillin, ampicillin, erythromycin, *Bacillus cereus*, *Bacillus* sp., *Enterococcus avium*, *Enterococcus durans*, *Enterococcus faecium*, methicillin-susceptible *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Streptococcus* groups C, F, and G, *Streptococcus milleri*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Clostridium difficile*, *Clostridium perfringens*, and anaerobic cocci.

Statistical methods. Descriptive statistics of these raw values of the $\log_2$ *cMICs* were performed for the eight antimicrobial agents across the 17 species studied. Before performing multivariate analysis, the $\log_2$ *cMICs* were standardized by subtracting the mean value of each antimicrobial agent from each value and by dividing each difference by the standard deviation of that particular antimicrobial agent. Pearson product-moment correlation coefficients for the eight pairs of antimicrobial agents were also calculated (see Table 2).

The multivariate modeling of the relationship between antimicrobial agents was performed by the factor analysis and PCA by using PROC FACTOR and PROC PRINCOM in SAS (SAS Institute Inc., Cary, N.C. [21a]). The information that covers the different factors can be tested by the variance that is maintained. The eigenvalues of the correlation matrix were calculated to estimate the variance (see Table 3), and thus the information contained by each component. A selection of the first three components kept 81.5% of the total variance. This means that 81.5% of the information contained in the eight initial variables is captured with the first three variables. Three components were considered sufficient to keep a sizable amount of information and resulted in a clear graphic representation in three dimensions (see Fig. 1). The three principal components were interpreted by using the factor pattern

TABLE 2. Pearson product-moment correlations between eight antimicrobial agents

Antimicrobial agent	Correlation							
	DAP	VAN	TEI	CLT	IMI	GEN	CIP	RIF
DAP	1.00000							
VAN	−0.22536	1.00000						
TEI	−0.14298	0.94675	1.00000					
CLT	0.24367	0.38014	0.46298	1.00000				
IMI	0.01055	−0.13548	−0.02193	0.57061	1.00000			
GEN	−0.02428	−0.25024	−0.26248	0.39142	0.58497	1.00000		
CIP	−0.34984	0.55885	0.41554	0.16252	−0.32376	−0.00706	1.00000	
RIF	−0.22822	0.66797	0.50566	0.37124	−0.03487	0.04752	0.70268	1.00000

TABLE 3. Eigenvalues of the correlation matrix and information contained in the different factors

Factor	Eigenvalue[a]	Proportion[b]	Cumulative information[c]
1	3.2163	0.4020	0.4020
2	2.0863	0.2608	0.6628
3	1.2142	0.1518	0.8146
4	0.7939	0.0992	0.9138
5	0.3180	0.0397	0.9536
6	0.2306	0.0288	0.9824
7	0.1193	0.0149	0.9973
8	0.0214	0.0027	1.0000

[a] Initial factor method, principal components; prior communality estimates, 1.
[b] Information contained in each factor.
[c] Cumulative information explained by the factor and the previous ones (proportion of the total variance).

matrix and applying a subsequent orthogonal axis rotation (Varimax type) (see Table 4). This rotation permits an easier presentation of the graphic results without affecting the interrelationships among them.

RESULTS

Base-2 logarithms of the corrected MIC_{90} ($\log_2$ *cMIC*) for the different species and antimicrobial agents considered for analysis are presented in Table 1. Pearson product-moment correlations for the eight paired antimicrobial agents are displayed in Table 2. A high level of correlation was observed between VAN and TEI (0.94675), while the next highest positive correlations were between CIP and RIF (0.70268) and between VAN and RIF (0.66797). On the other hand, DAP was not strongly correlated with any of the tested antimicrobial agents, and the correlations of DAP with VAN and TEI were insignificant (−0.22536 and −0.14298, respectively).

The numerical results (rotated factor pattern matrix) of the factor analysis on DAP, VAN, TEI, CLT, IMI, GEN, CIP, and RIF are presented in Table 4. The first principal component (factor 1) was dominated by VAN, TEI, and RIF (0.94344, 0.92792, and 0.72127, respectively). The second principal component showed strong effects from IMI, GEN, and CLT (0.87922, 0.86126, and 0.68870, respectively). DAP stood out alone in the third principal component (0.83983).

The first three principal components are represented in Fig. 1, which shows graphically the numerical results presented in Table 4. There appeared to be three separate clusters of the antimicrobial agents. In the foreground and occupying the lower octants of the graph, VAN, TEI, CIP, and RIF formed the first cluster. This cluster is reflected by the first principal component. The second cluster consisted of CLT, IMI, and GEN on the right side of the graph. This cluster is indicated by the second principal component. Finally, the third cluster, represented by the third principal component in the middle-top of the graph, had only DAP as a member. The factor analysis indicated that DAP did not cluster with the other seven antimicrobial agents. In other words, DAP did not assemble with the glycopeptides (VAN, TEI) or with CLT, IMI, GEN, CIP, and RIF in relation to the microorganisms studied (staphylococci, *E. faecalis*, *Listeria* species, group A and B streptococci, and the naturally vancomycin-resistant species *Leuconostoc*, *Lactobacillus*, and *Pediococcus*).

DISCUSSION

The representation in Fig. 1 groups antimicrobial agents in different spatial clusters. The more similar the chemical structure—and, thus, the more similar the mechanism of action—the closer two antimicrobial agents are in space. VAN and TEI, both glycopeptides, are almost together. Another observation from the graph is that the beta-lactams and aminoglycosides evaluated are also close together; glycopeptides, quinolones, and RIF, whose extended usage started in the 1980s, are in another cluster. Beta-lactams and aminoglycosides have been widely used antibiotics over the last three decades. The ability of multivariate techniques to delineate the impact of antimicrobial usage on resistance patterns has previously been suggested by Hunter et al. (16). Applying multivariate techniques, those investigators found a high degree of correlation between the activities of methicillin and GEN against strains of

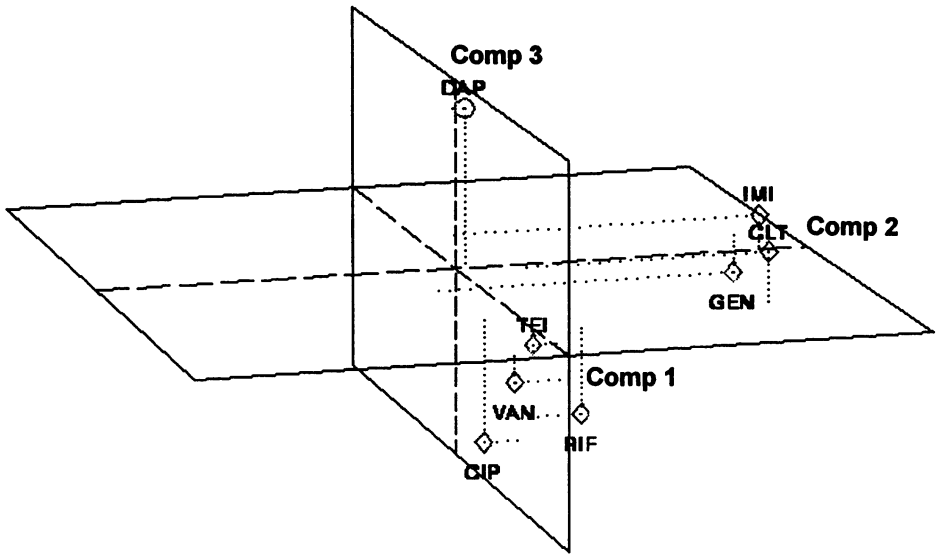

FIG. 1. Three-dimensional scatter plot of the relationships among the eight antimicrobial agents. This is a graphic representation of the three first principal components (Comp) on the basis of the data in Table 4.

TABLE 4. Rotated (Varimax) factor pattern matrix of the eight antimicrobial agents

Antimicrobial agent	Factor 1	Factor 2	Factor 3
DAP	−0.02301	0.03207	0.83983
VAN	0.94344	−0.12762	−0.14628
TEI	0.92792	−0.07342	0.03332
CLT	0.59217	0.68870	0.27374
IMI	−0.03774	0.87922	0.16705
GEN	−0.20413	0.86126	−0.21420
CIP	0.57500	−0.09744	−0.63906
RIF	0.72127	0.12285	−0.44643

Staphylococcus haemolyticus and postulated that this species underwent similar evolutionary resistance pressures for these two drugs. It is noteworthy that DAP appears alone in the spatial characterization described in the current study. Some of the features that could account for the differences observed in the present analysis with DAP are its lipopeptide structure (9), its membrane-targeted mechanism of action (1, 2), and its activity against VAN-resistant gram-positive organisms (6, 7, 22). DAP is a cyclic decapeptide linked by a tripeptide to a fatty acid chain (9). It disrupts the electrochemical gradient of the bacterial membrane (1, 2), impairing the transport of metabolic precursors and, indirectly, synthesis of the cell wall. Its structure and mechanism of action are very different from those of the glycopeptides (3).

Results from multivariate analysis techniques have obvious limitations. They depend critically on the data from which they are generated and the conclusions may not be extrapolated to other collections of data, at least in a quantitative manner. This is why the results presented here can be applied only to the eight antimicrobial agents tested and the species noted. Also, the conclusions cannot be extended beyond the limits of the methods used. Multivariate analyses measure differences in in vitro profiles but ignore efficacy; a very unique drug as depicted by a multivariate technique could be absolutely ineffective in vivo. In the present study, in which only in vitro data were considered, no clinical assumptions can be made. This is why PCA and factor analysis should be considered only as additional tools for use in the process of analyzing susceptibility data. The sequential application of multivariate analysis over a certain bacterial population may also prove to be useful in assessing the development of antimicrobial resistance over time. However, only one application of this analysis to one data set would not be able to assess changing drug resistance patterns.

PCA has already been applied in biology and medicine, in areas such as neurology, psychology, psychiatry and psychometrics, biochemistry, transplantation, genetics, and clinical trial design. Nevertheless, it has rarely been applied in microbiology. The use of multivariate techniques is a different approach to the way in which the in vitro activities of antimicrobial agents are studied and can add information on the interrelations among drugs and species. The prospective application of these statistical methods in microbiology appears to be promising. Multivariate techniques such as PCA, factor analysis, principal coordinate analysis, or cluster analysis could be used to add information about the complex interrelationships among antimicrobial agents, microorganisms, and perhaps, even the host. The sequential application of multivariate methods over evolving bacterial populations could also add information to provide a better understanding of the evolving resistance trends and the mechanisms responsible for them. In new drug discovery, these techniques could be added as a tool in the early development phases in order to help identify similarities or differences between the in vitro activities of new drug candidates. The scope of future applications of multivariate techniques in microbiology, antimicrobial chemotherapy, and drug research appears to be broad, and further studies are certainly warranted.

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